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Lexatumumab (TRAIL-receptor 2 mAb) induces expression of DR5 and promotes apoptosis in primary and metastatic renal cell carcinoma in a mouse orthotopic model ☆

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Abstract

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces apoptosis in a variety of tumorigenic and transformed cell lines but not in many normal cells. Hence, TRAIL-agonist compounds have the potential of being excellent cancer therapeutic agents with minimal cytotoxicity. Here, we examine the efficacy of the TRAIL-receptor 2 agonist, lexatumumab (Human Genome Sciences, Inc., Rockville, MD), and identify molecular pathways that differentiate between lexatumumab-sensitive and lexatumumab-resistance renal cancer cells. In an orthotopic metastatic mouse model, we first demonstrate that lexatumumab was effective in reducing the tumor burden of primary and metastatic lexatumumab-sensitive xenografts. We demonstrate that lexatumumab-sensitive cells were capable of triggering both the extrinsic and the intrinsic apoptotic pathways as demonstrated by caspase 8 and caspase 9 activations, respectively, after treatment with lexatumumab. In addition, expression of c-FLIP(L) protein, an important regulator of TRAIL-induced apoptosis, decreased, while expression of the TRAIL-receptor 2, DR5, increased. This study serves as a pre-clinical model for using TRAIL-like therapies for patients with advanced RCC.

Keywords: TRAIL; DR5; c-FLIP(L); Lexatumumab; Metastasis; Orthotopic; Renal cell carcinoma

1. Introduction

Renal cell carcinoma is the most common malignant tumor of the kidney, accounting for 85% of all renal cancers. Almost 20–30% of patients with newly diagnosed renal cell carcinoma have metastatic disease at presentation and their median survival is 6 to 8 months, accounting for over 12,000 deaths in the United States annually [1,2]. Despite advances in various systemic therapies (e.g. immunotherapy, dendritic vaccine therapy, sorafenib and sunitnib),

Abbreviations: TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; RCC, renal cell carcinoma; FADD, Fas-associated protein with death domain; DED, death effector domain; DISC, death-inducing signaling complex; DD, death domain; c-FLIP, cellular FLICE-like inhibitory protein; c-FLIP(L), long form of c-FLIP; c-FLIP(s), short variant of c-FLIP; DR, death receptor.

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treatment of metastatic renal cell carcinoma remains a challenge with treatment responses ranging from 10–30% with only limited durability of response and minimal improvement in survival [2,3]. Therefore, novel treatment strategies are necessary to improve the survival from metastatic RCC.

Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL/Apo2L) is a promising anticancer agent due to its ability to induce apoptosis in many cancer cell lines, with minimal to no effect on most normal cells [4,5]. TRAIL interacts with specific death domain receptors, DR4 and DR5, to induce intracellular cytoplasmic formation of the DISC (Death Inducing Signaling Complex) [5–9]. DISC formation involves the recruitment of caspase-8 via adaptor proteins (FADD, TRADD, and RIP) to the death domain of the activated receptor [10,11]. Activated caspase 8 triggers the extrinsic apoptotic pathway by directly activating effectors such as caspase 3 and caspase 7. Caspase 8 can also initiate the intrinsic apoptotic pathway through the activation of Bid [12,13]. Both pathways lead to the activation of caspase 3 and eventual apoptotic cell death [12]. A key inhibitor of death receptor signalling is c-FLICE-like inhibitory protein (c-FLIP). c-FLIP shows a high level of homology to caspase-8 and caspase-10 but has no protease activity and prevents the formation of a competent DISC by binding to the FADD adaptor protein and competing off caspase-8 [14–17]. Although
c-FLIP is expressed as multiple splice variants, two main forms are expressed at the protein level: c-FLIP short form (c-FLIP(s)), which is 28 kDa in size and contains two death effector domains, and c-FLIP long form (c-FLIP(L)), which is 55 kDa in size and has two death effector domains and an inactive caspase-like domain [16,18–20]. More recently, a third isoform c-FLIP family, c-FLIP(r), has been discovered [21]. c-FLIP(r) is a splice variant of c-FLIP(s), which contains an open reading frame stop codon, and lacks the carboxy terminal amino acids as compared to c-FLIP(s). We have previously demonstrated that expression of the anti-apoptotic gene, c-FLIP(L), is necessary and sufficient to maintain resistance to TRAIL-induced apoptosis in prostate cancer cells [22]. Here, we show that the TRAIL-receptor 2 agonist, lexatumumab, promotes DR5 protein expression and reduces c-FLIP(L) protein level.

Although the efficacy of TRAIL has been examined in subcutaneous and orthotopic xenograft models [23–28] to our knowledge, its efficacy has not been examined in an orthotopic metastatic model. In addition, the efficacy of the TRAIL-receptor 2 agonist, lexatumumab, which is the only TRAIL-receptor agonist currently in clinical trials, has not been examined in an orthotopic metastatic model. We show that lexatumumab exerted activity as a single agent against RCC tumors *in vivo*, supporting an apoptosis-based biological approach for treatment of locally advanced and metastatic RCC.

Lexatumumab [29,30] is a fully humanized agonistic monoclonal antibody that specifically binds to DR5 receptor, and is currently in phase I/II clinical trials in patients with advanced malignancies. Although initial clinical trials using lexatumumab in advanced malignancies have been promising, some patients are resistant to the pro-apoptotic effects of lexatumumab. The mechanism of resistance to lexatumumab induced apoptosis, which is currently in clinical trials, has not been studied. In our study we demonstrate that lexatumumab is effective for treatment of primary and metastatic lexatumumab-sensitive xenografts. In addition, we demonstrate molecular pathways which differentiate between lexatumumab-sensitive and -resistant RCC cells.

2. Materials and methods

2.1. Reagents

Horse radish peroxidase-conjugated secondary antibody Ab (goat-anti-mouse, goat-anti-rabbit, goat-anti-rat antibodies) and DR4 Ab were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Caspase 8 Ab, Caspase 9 Ab and Caspase 3 Ab were obtained from Cell Signaling (Beverly, MA). Monoclonal Anti-FLIP Ab (Dava II) and DR5 Ab for Western blot were obtained from Apotech Corp. (San Diego, CA). Anti-FLIP Ab and DR5 Ab for Immunohistochemistry were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). GAPDH Ab and BID Ab were from Abcam, Inc. (Cambridge, MA).

2.2. Cell culture

All cell culture materials were from Cellgro (Herndon, VA) and plastic-ware was from Becton Dickinson Labware (Bedford, MA). A-498 and 786-O were obtained from the American Type Culture Collection (ATCC, Manassas, VA). SN12-PM6 was supplied by Dr. I.J. Fidler (MD Anderson Cancer Center, Houston, TX). A-498 was grown in Eagle's Minimum Essential Medium (EMEM). SN12-PM6 was grown in DMEM medium with MEM Vitamin Solution and Nonessential Amino Acid Solution from Cellgro (Herndon, VA).